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E D I T O R I A L

UNFAIR HOSPITAL COMPETITION.

THERE seems to be no end to the inroads being made on the business of retail pharmacy. We have already spoken out against both the supermarket and the physician-owned pharmacy. The latest invasion of the retail field is potentially worse—that by the hospital.

On the West Coast a pharmacy operated by a hospital has made an open attempt to encourage the public to bring its prescriptions to the hospital to have them filled. One need not be an out-patient of the hospital to have a prescription filled and the prescribing physician does not have to be on the hospital staff. The hospital has announced publicly that this is an innovation solely for the purpose of increasing its revenue.

At first glance there are those who might say "Well, why not? Is the hospital pharmacy not as well equipped to do this as the corner drug store?" Such superficial judgment loses sight of the fact that the hosptial is a tax-free institution and in most cases is the recipient of state aid. It is manifestly impossible for the retail pharmacist to meet such unfair competition. The truth is that part of the tax which he himself must pay goes to assist his competitor force him into bankruptcy. Many of our colleges of pharmacy have "model" drug stores on their premises and manufacturing equipment used for teaching purposes. They, too, are tax-free institutions and it would be grossly unfair for them to manufacture and sell drugs to the community at large. A few colleges do fill prescriptions for their own students but this is similar to the service rendered by the hospital pharmacy for patients in the hospital.

By and large, pharmacists and boards of pharmacy have been extremely, if not excessively, tolerant of the practice of pharmacy in the hospital. In many states the retail pharmacy is subjected to much more stringent requirements than is the hospital pharmacy. In many hospitals pharmacy is actually practiced by unlicensed personnel without professional supervision. It is doubtful whether our profession should permit this in the public interest, but many of

us have hesitated to saddle the smaller hospitals with the added costs which a staff pharmacist and adequate equipment would entail.

The solution of this problem of unfair hospital competition seems obvious. Strongly worded resolutions at conventions will do little if any good. The answer lies in retail pharmacists banding together and insisting that hospitals who indulge in the conduct of a business be assessed and taxed as are other businesses. State appropriations should also be blocked wherever possible by every available means of legislative pressure.

It is inconceivable that the unfairness of this situation be ignored. In many respects retail pharmacy is fighting for survival but most retailers are not yet aware of just how serious the situation is. Unfair hospital competition is but one aspect of a very complex problem. One thing is certain—retail pharmacists must discontinue their internal strife and distrust of each other and direct their collective thought and energy towards meeting threats far worse than that posed by a fellow pharmacist a few blocks away. Organized pharmacy has a tough job to do and difficult days lie ahead. With unity and effort we can succeed. Above all we must insist on the blocking of unfair competition regardless of its source.

L. F. Tice

NATURAL ANTIBODIES IN RABBITS, GUINEA PIGS AND HAMSTERS

By Louis Gershenfeld and Howard Cravetz *

IT is well known that many animals possess antibodies against a variety of antigens. These "natural" or "normal" antibodies are differentiated from induced antibodies by several characteristics. First, they occur in the serum of animals which were never artificially immunized, and were unable to receive colostrum from the mother, e.g. chicks. Secondly, they appear despite the lack of any apparent environmental stimulus, e.g. infections, even though rigorous control measures are enforced. Also, the titers found are comparatively low (5) (11) (28).

Landois (19), in 1875, did extensive blood transfusion research which led him to his report of cross-agglutination and hemolysis of the blood of various animal species. Ludke (23) performed similar work on hemolysins. Kolle, et al. (18) found that rabbits, guinea pigs, sheep, horses and cattle possessed natural titers of *V. cholera* agglutinins.

Burgi (5), in 1907, tested normal rabbit sera against suspensions of *V. cholera, S. typhosa, E. coli, P. avisepticum, M. pyogenes aureus* and *P. vulgaris.* Gibson (11), in his survey, included a larger number of members of the intestinal flora, such as *Sh. paradysenteriae, Sh. shigae, S. enteritidis, S. paratyphosa* and *S. schottmuelleri.* In contrast to Landois, Gibson found no titer against *V. cholera.* Both Gibson and Burgi found most animals low in agglutinins for almost all organisms. Burgi concluded that in general, animals high in titer against one organism were high against all organisms. He attributed the phenomenon to an aspecific agglutinin, but more recent workers have found this to be incorrect (15) (21) (22).

It appears at present that the kinds of antibodies found are limited only by the variety of antigens available for testing. One worker, Walsh (32), even found a spermatocidal antibody in guinea pig serum which killed the same animal's sperm in vitro.

Reports on natural antibodies are frequently contradictory, e.g. the Gibson-Landois discrepancy regarding *V. cholera.* Lovell (22)

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reported that rabbits seldom show the presence of either H or O *Salmonella* agglutinins. Mackie and Finkelstein (24) (25), however, after testing a wider range of organisms, reported that they found definite agglutination throughout, although the agglutinins were at low titers.

The essential fact remains that natural antibodies do exist, and their origin as well as their distribution is still a matter of controversy. This dispute arises from two divergent general theories regarding the stimulus for production of the so-called natural antibodies. It is a familiar observation in immunological work that young animals undergo a period during which no antibody production is demonstrable, whether naturally or artificially induced. At an age characteristic for the species, however, naturally occurring antibodies appear in the blood stream and progressively increase in titer to a constant level. At the same time it becomes possible to induce the production of antibodies artificially. Kershaw (17), Duran-Reynals (6), and Bailey (3) have reported this in chicks, Ambrus, et al. (2) in chicks and rats, and Friedberger and Bock (10) have graphed the rise and decline of natural hemolysins in both humans and rabbits (see Discussion). In investigating the age threshold for antibody production against cancer tissue, Ambrus (1) reported that Ehrlich mouse carcinoma, normally viable only in mice, was grown in rats up to the age of three weeks. The onset of absorption of the tumor was concurrent with the appearance of antibodies against sheep cells, injected simultaneously with the cancer.

The results obtained by these workers tend to support the classic views of Landsteiner (20) and Hirszfeld (14). Hirszfeld's theory of "Serogenesis" regards antibodies as biochemical organs which mature at definite stages in the animal's development, enabling it to produce antibodies despite the absence of external antigens.

In direct contradiction to the above theory of internal stimulus are several theories regarding external stimuli, proposed by eminent workers, and borne out by valid, reproduceable data. Bailey (4) and Forssman (8) (9) reported extensive work on rabbits, in which it was shown that several heretofore neglected routes of immunization are possible. Forssman, by feeding or injecting certain green foods, and Bailey, by intranasal injection with *B. lepisepticum,* demonstrated the subsequent formation of heterophilic sheep hemolysin by the animals. Ramon, Richou and Djourichitch (30) reported the formation of staphylotoxin in guinea pigs subsequently found to have

suffered subclinical staphylococcal infections, as a result of poor sanitation. Powell (*27*), Ramon and Richou (*29*) and Ingalls (16) have supported this viewpoint with their findings concerning intestinally absorbed antigens. Ehrlich (*7*), Wright and Clark (33) and Reymann (31) have shown the significance of colostrum as a source of antibodies in young animals.

In view of all the complex and often conflicting data, much of which is doubtful, an opinion regarding the nature of normal antibodies can be reached only by analysis and a critical evaluation of the work done. The present status of the problem seems to be a viewpoint which encompasses all of the above theories, arranging them in a logical pattern into a "combined theory", as shown below:

(A) Genetically, the particular antibody-forming mechanisms may or may not be present in the animal.

(B) Those which *are* present mature and function at a certain stage in the animal's development.

(C) Of the mature mechanisms present, some form antibodies without apparent external stimuli, others respond to unknown or undetected stimuli, and still others respond only to artificially administered antigen or to active infections.

The question to be answered is, "Are the supposedly normal antibodies present due to the maturation of the antibody system's manufacturing natural antibodies according to a genetic pattern; or are they merely the normal response of a newly-matured antibody mechanism to the variety of potentially antigenic materials present in the animal's environment?"

Purpose

The present study was undertaken to further investigate the problem of natural antibodies from two points of view; first, by a general survey of the range of natural antibodies in rabbits, guinea pigs and hamsters (concerning hamsters, the literature is still sparse), and second by an approach to the question of source of the antigens responsible for their presence. Two main problems are considered; one, the maintenance of animals in an environment as free as possible from known antigenic stimuli; and the other, the removal of antibodies from sera by treatment with homologous animal tissues, a possible site of concentration of any antigenic material derived from the surroundings.

Experimental

Materials

1. Isotonic solution of sodium chloride (0.9%), U. S. P., herein referred to as "isotonic saline".

2. Sera: the inactivated sera of normal New Zealand white and Hare Brown rabbits, English short-haired guinea pigs and Syrian golden hamsters, prepared as described under "Methods".

3. Blood cells: the blood cells of the following animals, prepared as described under "Methods": rat, sheep, horse, cow (beef), dog, rabbit, hamster, guinea pig, mouse, chicken, pigeon and man.

4. Sodium citrate solution. 3.5%.

5. "Febrile" antigens: *Proteus Ox-19, Salmonella typhosa O-(I, IX, XII), Salmonella typhosa H (d), Paratyphoid A (I II, XII, a),* and *Paratyphoid B (I, IX, XII, b).* All were commercial diagnostic antigens.[1]

6. Test tube method antigens: suspensions of the following organisms[2] were prepared as described under methods: *P. morgani, Sh. alkalescens, E. freundii, Ser. marcesens, P. vulgaris, Sh. paradysenteriae sonnei, N. catarrhalis, C. hoagii, G. tetragenus, Sar. lutea, K. pneumoniae, Ps. aeruginosa, Sh. paradysenteriae flexner, M. pyogenes aureus 209, B. brevis, L. arabinosus, B. mesentericus, B. megatherium, C. diphtheriae,* and *B. subtilis.*

7. Organ homogenate: suspensions in isotonic saline of the following organs of a rabbit, guinea pig and hamster, respectively: heart, liver, lung, spleen and kidney. Contents of the small intestine were included in each of the three homogenates.

8. Animals: The rabbits and guinea pigs employed were obtained from Rockland Farms. Two species of rabbits, Hare Brown and New Zealand Whites, were used, the individuals all being females of about three kilograms. The guinea pigs were adult English short-haired of both sexes. The authors were advised that these animals never had access to greens or fresh vegetables, but had subsisted from birth entirely on Rockland[3] pellets and water. The hamsters were

1. Supplied by the Lederle Laboratories and Parke, Davis & Company.
2. The cultures used were obtained from the Bacteriology department of the Philadelphia College of Pharmacy and Science, Sharp and Dohme, Inc., and the American Type Culture Collection.

Syrian Golden hamsters, all males, of approximately 100 grams, and previously had been fed greens as well as commercial pellets.

All animals were housed in air conditioned quarters at 21° C., in draft-free cages. Several advantages are thus presented; dust and pollen were filtered out, there were no appreciable temperature variations, such as would favor respiratory infections, and this temperature (21° C.) is considered the optimum for raising animals at their peak of vigor.

All cages were washed weekly and sterilized in a 5% phenol solution. Preventive medication was used to combat ear canker in the rabbits. The experiment of Ramon and Richou (29) illustrates the importance of such hygienic measures in experiments of this nature.

The diet of all animals was rigidly controlled. At no time were the rabbits or guinea pigs fed greens; their food (and that of their ancestors for generations back) from the time of birth has been Rockland Rabbit Ration [3] and Guinea Pig Diet,[3] water ad lib and access to salt spools. The two diets are identical, except that guinea pig diet has no salt and contains vitamin C. These foods contain the full complement of vitamins, minerals and caloric value required by the animals. The hamsters were fed Rockland [3] Rat food in our laboratory, but had previously been fed greens.

Methods

1. *Preparation of sera:*—Guinea pig blood was obtained by heart puncture, hamster blood by severing the jugular veins, and rabbit blood was obtained from arteries or veins from the ear. The blood samples were obtained under aseptic conditions, allowed to clot in sterile test tubes, refrigerated at 4° C. for thirty minutes, and then centrifuged for five minutes at 1500 R. P. M. Sera were removed aseptically and placed in sterile tubes. Inactivation of complement was performed at 56° C. in a water bath for thirty minutes. All sera were then stored at 19° C. until needed.

2. *Preparation of blood cell antigens:* Blood was obtained aseptically from the respective animals and placed into sterile sodium citrate (final concentration of citrate was 0.35%), mixed, and centrifuged for five minutes at 1500 R. P. M. The supernatant plasma was discarded, replaced by sterile isotonic saline, and the blood cells

3. Products of Arcady Farms Milling Co., 223 W. Jackson Blvd., Chicago, Ill.

were again mixed and centrifuged. The cells were washed three times and were kept a maximum of three days, since hemolysis began to appear thereafter. Shortly before use, a 3% suspension of cells in sterile isotonic saline was prepared.

Considerable difficulty was encountered with dog cells; they were found to exhibit some degree of hemolysis in all instances, even during a fifth washing in isotonic saline. This, however, did not seem to alter the agglutination in any way.

Hamsters were found to be very susceptible to intrapericardial tamponade when heart punctures were performed. Thus consecutive punctures of the same animal were considered impractical, and a complete bleeding, through the jugular veins, was therefore employed.

3. *Preparation of organ homogenates:* The heart, lung, liver, spleen and kidney of each animal were excised aseptically, placed in a sterile beaker, finely minced with sterile scissors, and washed three times with sterile isotonic saline, to remove blood. The small intestine was excised and the contents added to the organ mass. The homogenates were prepared immediately prior to use.

4. *Absorption of sera:* Each of the three sera was mixed with the homogenate of the homologous animal and incubated at 37° C. for two hours. The serum was separated from the solid elements by centrifuging for fifteen minutes at 2,000 R. P. M. and decanting.

5. *Preparation of bacterial antigens:*

(a) A 24 hour culture of the organism was grown in a four-ounce bottle on a slant of 3% nutrient agar medium.

(b) A few cubic centimeters of sterile isotonic saline were poured over the slant, following which it was shaken for fifteen seconds. The resulting suspension was aseptically transferred to a sterile test tube.

(c) The suspension was diluted with sterile isotonic saline to conform in turbidity with a similar tube, prepared to contain an estimated two billion bacteria per cubic centimeter. The suspension was stored at 4° C.

6. *Bacterial Agglutination Tests:* Where commercial antigens were available, slide agglutinations were performed. In the case of those bacterial antigens not readily available, antigen suspensions, prepared as described above, were employed.

A. *Slide Agglutination Test:* Large glass slides, thoroughly clean, were marked off into inch and a half squares with a colored pencil. The serum to be tested was pipetted in amounts of 0.08, 0.04, 0.02, 0.01, and 0.005 cc. into each row of squares on the slide, from left to right in decreasing amounts, and 0.03 cc. of antigen was pipetted into each serum square (see chart).

Rabbit	.08 cc. serum	.04 cc. serum	.02 cc. serum	.01 cc. serum	.005 cc. serum	.03 cc. saline
Serum	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen
Guinea Pig	.08 cc. serum	.04 cc. serum	.02 cc. serum	.01 cc. serum	.005 cc. serum	
Serum	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	
Hamster	.08 cc. serum	.04 cc. serum	.02 cc. serum	.01 cc. serum	.005 cc. serum	
Serum	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	.03 cc. antigen	

The relative proportions of antigen to serum thus approximated those of the usual tube test, i.e. 1:20, 1:40, 1:80, 1:160 and 1:320. A similar test was performed for each of the five slide antigens. The contents in the squares in each row of serum-antigen mixture were mixed with a toothpick and the slide was gently rotated by tilting about twenty times during the three minute period following the addition of the antigen. The agglutinations were then noted macroscopically and recorded.

B. *Tube Agglutination Test:* Five rows of 9 x 75 mm. lipless test tubes were employed. The number of tubes in each row was equal to the number of different antigens being tested plus one isotonic saline-antigen control tube. Each tube contained 0.3 cc. isotonic saline. Three tenths cc. of the serum being examined was placed in each tube of the first row; then half of this solution was transferred to the corresponding tube in the second row. This was continued until the fifth row, the last 0.15 cc. being discarded. The first row now contained 1:2 serum dilution, the second, 1:4, the third, 1:8, the fourth, 1:16, and the fifth, 1:32. Three tenths cc. antigen wes added to each tube and the entire rack was shaken. After two hours incubation at

37° C., the tubes were centrifuged at 1,500 R. P. M. for 1½ minutes, and readings were recorded. The following procedure was used in reading results:

A tube, held loosely at the top, was suspended before a bright reflecting surface. The bottom of the tube was struck one to three sharp blows, so that the entire contents was thrown upwards (the actual number of blows was determined by the number required to resuspend the control tube contents).

A value of plus four was given when the antigen mass broke up into one to three large clumps; a value of plus three was given when three to ten clumps appeared; plus two was assigned to rough granulation, and plus one for not quite as heavy, but well-defined agglutination. Tests resulting in plus-minus values were repeated several times, and if this status persisted, they were assigned a positive value. The assigned values are arbitrary. Positive and negative values only are considered in the discussion.

Findings

As shown in table 1, the different bacteria agglutinated were very few in number. Only three out of the twenty organisms investigated were agglutinated, and even these were not very marked. The titers were: for rabbit—*Serr. marcessens,* 1:32, *N. catarrhalis,* 1:8; for guinea pig—*B. subtilis,* 1:4; for hamster—*N. catarrhalis,* 1:16. All three organisms are air-borne non-pathogens.

Rabbit serum agglutinated nine of the thirteen different kinds of blood cells, guinea pig serum agglutinated ten, and hamster serum nine (see table I).

Absorption of the sera with homologous organ homogenates removed agglutinins in about one third of the positive sera (eleven out of thirty one revealed removal of agglutinins). In two instances this treatment added some hemolytic factor to the serum which was diluted out at 1:16, so that agglutination was again noted.

The agglutination spectrum of normal rabbit serum and guinea pig serum was found to agree quite closely with that described by Preisz (28) in Hungary (see table III).

It was not possible to make a comparison of the bacterial agglutinins found here with those of other workers, since the strict control measures used here were not used by them.

TABLE I

BACTERIAL AGGLUTINATION BEFORE AND AFTER SERUM ABSORPTION

(Tube Method)

Antigen	Rabbit		Guinea Pig		Hamster	
	Before	After	Before	After	Before	After
P. morgani	—		—		—	
Sh. alkalescens	—		—		—	
E. freundii	—		—		—	
Serr. marcescens	+	+	—		—	
P. vulgaris	—		—		—	
Sh. sonnei	—		—		—	
N. catarrhaliis	+	+	—		+	—
C. hoagii	—		—		—	
G. tetragenus	—		—		—	
Sar. lutea	—		—		—	
K. pneumoniae	—		—		—	
Ps. aeruginosa	—		—		—	
Sh. paradysenteriae	—		—		—	
M. pyog. aureus 209	—		—		—	
B. brevis	—		—		—	
L. arabinosus	—		—		—	
B. mesentericus	—		—		—	
B. megatherium	—		—		—	
C. diphtheriae	—		—		—	
B. subtilis	—		+	—	—	
	(Slide Method)					
S. paratyphosa	—		—		—	
S. schottmuelleri	—		—		—	
S. typhose O	—		—		—	
S. typhosa H	—		—		—	
Proteus OX-19	—		—		—	
BLOOD CELL AGGLUTINATION BEFORE AND AFTER SERUM ABSORPTION						
	(Tube Method)					
Sheep cells	—		+	+	+	+
Horse cells	+	—	+	+	+	—
Beef cells	—		+	+	+	—
Chicken cells	—		+	—	+	+
Pigeon cells	+	—	—		—	
Guinea Pig cells	+	+			+	+
Rat cells	+	+	+	+	+	+
Mouse cells	+	+	+	+	+	+
Hamster cells	+	—	+	+		
Human cells	+	+	+	+	—	
Rabbit cells			+	+	+	—
Dog cells	+	—	+	—	+	+

TABLE II

COMPARISON OF DATA OF PAST WORKERS ON BACTERIAL AGGLUTININS IN NORMAL SERA

Organism**	Rabbit			Guinea Pig		
	Bürgi (5)	Gibson (11)	Mamlock (26)	Bürgi	Gibson	Mamlock
V. cholera	1:1	0	1:2	0	1:16	1:1
Sh. dysenteriae	1:128		1:8	1:4		1:4
S. karatyphosa		0	1:1		0	
S. schottmuelleri	±	0		0	1:16	0
E. coli	1:1	0	1:4	1:2	1:8	1:2
S. typhimurium	0		1:2	?	1:8-1:16	0
Proteus vulgaris	1:2		1:4	0		0
Ps. aeruginosa	?	1:16	1:4	?	1:8-1:16	0
S. aureus	0		1:8	0		0
S. suipestifer	0		1:1	0		1:2
Proteus vulgaris OX-19		0			0	
S. typhosa		1:32	1:4		1:8-1:16	1:4
K. pneumoniae		1:128			1:32	
B. dysenteriae Y		1:128			1:16	
S. enteritidis		0			1:8	
Sh. shigae		1:8			1:8	
P. morgani					1:18	
B. anthracis			1:16			1:8
Avian cholera			1:2			1:2

** The nomenclature employed is that of the authors.

TABLE III

COMPARISON OF DATA ON HEMAGGLUTININS

Source of Erythrocytes	Preisz (28)	Gershenfeld-Cravetz	Preisz	Gershenfeld-Cravetz
Human	—	+	+	+
Horse	—	+	+	+
Sheep	—	—	+	+
Chicken	—	—	+	+
Guinea Pig	+	+		
Pigeon	+	+	+	—
Rabbit			+	+

(+ denotes agglutination)

Discussion

From the data obtained in this study it is apparent that none of the animals used revealed the presence of any appreciable range of bacterial agglutinins as compared to that reported by workers in the literature (5) (11) (26). To account for these differences, several explanations are possible:

1. The technics and experimental conditions employed were not identical.

2. Different strains of the same animals were used.

3. Assuming some "natural" antibodies to be in reality externally stimulated, stimuli present under some experimental conditions are lacking in others.

The technic of testing used here was a standard procedure employed by other workers (with the exception of the slide agglutination tests); inactivation of complement was carried out in the accepted manner, and preservation by freezing is known to have no deleterious effect upon antibodies. The strains of animals chosen are strains used even a century ago in laboratories. The most obvious difference, then, is the absence of certain external antigenic stimuli in the present work. This is borne out by the fact that in blood cell agglutinations, performed under the same conditions and technic as for bacteria, the results were similar to those of past workers (28). More than likely, differences are due to the treatment and environment of the animals whose sera were tested.

A—Formerly, animals were obtained from farmers and other casual suppliers. Today they usually are obtained from a source which specializes in supplying clean, healthy animals of known heredity and history which have been raised under close veterinary supervision.

B—The scientific methods applied in the raising and maintenance of our animals minimizes the likelihood of their ever having been diseased (see animal maintenance, under Materials).

C—The diets fed here were rigidly controlled in such a way as to maintain a high vigor and avoid all known antigenic foods.

The uniformity of feeding and rearing minimizes the probability of the presence of disease or nutritional deficiency, as well as the probability that antibody production was stimulated by known external antigens.

No effort is made to correlate hamster data with that of rabbits and guinea pigs on the basis of the abovementioned conditions, inasmuch as the former had a different rearing and diet before reaching our laboratory. Hamsters were utilized because their increasing prominence as laboratory animals warrants a greater knowledge of their immunological response than is to be found in current literature. Haly (12) reports that they are good antibody formers against human blood cells. A further immunologic investigation of these animals, under controlled hereditary and invironmental conditions, is indicated.

Absorptions of the various sera with homogenates of liver, kidney, lung, spleen and heart and intestinal contents of the homologous animal failed to remove the agglutinins in the case of the sheep cells. This was also true for the majority of the agglutinins tested. It was noted that a low titer of hemolysin was acquired by the sera of rabbits and hamsters in this process, masking aggutinins until diluted out at 1:16, following which agglutination was again demonstrable. Exposure to the air during incubation may easily have resulted in the growth of common air-borne bacterial contaminants in the serum. Thus the removal of agglutinins for *B. subtilis,* one of the most ubiquitous laboratory contaminants, may be explained.

The finding regarding sheep cell agglutinins is of particular interest because it involves the well-known Forssman antigen. The serum of rabbits raised on Rockland diets showed no agglutination of sheep cells, whereas guinea pig serum demonstrated titers as high as 1:32, in some instances. Both are susceptible to the same diseases (though to different degrees), both had the same diet and the same environment; therefore an explanation of the antibody difference must be sought.

Forssman (8) showed that the heterophilic sheep cell antibody of rabbits is obtained from the greens they eat in nature. Bailey (4) showed that certain infections are capable of producing similar antibodies. Guinea pigs are Forssman-positive, i.e. their organs contain Forssman antigen (8).

From these considerations, the following deductions are presented:

1. Rockland rabbit and guinea pig food contains no Forssman antigen.

   (a) Otherwise the rabbit serum would contain sheep cell agglutinins.

2. The sheep cell agglutinins of the guinea pig are not heterophilic antibodies.

(a) Absorption of serum with an organ homogenate would have removed heterophilic antibodies, since both bacterial foci and Forssman antigen are present there.

(b) There can be no Forssman antibodies in a Forssman-positive animal, for this would kill him by a reverse passive anaphylactic shock.

Therefore, the following are concluded:

1. The so-called sheep cell antibodies sometimes found in rabbits are really heterophilic antibodies, such as were described by Forssman (8) and Bailey (4).

2. The anti-sheep cell antibodies always found in guinea pigs are not Forssman antibodies, but are probably true natural antibodies, since;

(a) No stimulus was present in the food.

(b) No stimulus by infection occurred.

Another noteworthy aspect of this study is the wide range of hemagglutinins evidenced by all three species tested, in contrast with the very small range of bacterial agglutinins. This difference does not lend support to one single theory of natural antibody formation over another, but favors the "Combined Theory" mentioned previously (see introduction). This apparent duality in the character of the antibody forming system leads to the hypothesis that two different types of antibodies are produced (regardless of their manifestations), in the sense that one is due to exogenous stimuli, e.g. bacterial infections, Forssman antigens or injected antigens, and the other is due to endogenous stimuli, e.g. the genetic pattern with which the animal is endowed by heredity.

Summary and Conclusions

1. The sera of twelve rabbits, ten guinea pigs and ten hamsters were tested for agglutinins against twenty different bacterial species and the blood cells of twelve different animal species.

2. Tests were performed by the tube agglutination procedure and by a rapid slide agglutination method.

3. Each serum was treated with a homogenate of organs of the homologous animal. Positive tests with non-treated sera were repeated, using treated (absorbed) sera.

4. Tables were presented showing a comparison of the findings of several workers in studies of normal antibodies in rabbits and guinea pigs.

5. A table is presented showing the findings of the authors' investigation of bacterial and blood cell agglutinins in rabbits, guinea pigs and hamsters.

6. Among animals maintained under certain controlled environmental and dietary conditions, relatively few bacterial agglutinins, as compared to the number of blood cell agglutinins were found.

7. The sheep hemolysins usually found in rabbits are heterophilic antibodies, whereas those of the guinea pig are true natural antibodies.

BIBLIOGRAPHY

(1) Ambrus, J., Ambrus, C., Harrisson, J. W. E., and Cravetz, H.: *This Journal 122,* 19 (1951).

(2) Ambrus, J.: Personal Communication.

(3) Bailey, C. E.: *Amer. Jour. Hyg. 3,* 370 (1923).

(4) Bailey, G. H.: *Amer. Jour. Hyg. 7,* 370 (1927).

(5) Burgi, E.: *Archiv. f. Hyg. 62,* 239 (1907).

(6) Duran-Reynals, F.: *Yale Jour. Biol. and Med. 12,* 361-398 (1940).

(7) Ehrlich, P.: *Z. Hyg. Infekt. kr. 12,* 183 (1892).

(8) Forssman, J.: *Biochem. Ztschr. 37,* 78 (1911).

(9) Forssman, J.: *Acta Path. and Microbiol. Scand. 23,* 145 (1946).

(10) Friedberger E. and Bock, G.: *Klin. Wchnschr. 8,* 1858 (1929).

(11) Gibson, H. J.: *J. Hyg. Camb. 30,* 337 (1930).

(12) Haly, J. G.: Personal Communication.

(13) Hirszfeld, L., Hirszfeld, H., and Brokman, H.: *J. Immunol. 9,* 571 (1924).

(14) Hirszfeld, L.: *Ergebn. Hyg. Bakt. 8,* 367 (1926).

(15) Hirszfeld, H., and Hirszfeld, L.: *Z. Immun. Forsch. 54,* 81 (1927).

(16) Ingalls, M. S.: *J. Immunol. 33,* 123 (1937).

(17) Kershaw, B. B.: *Yale Jour. Biol. and Med. 21,* 463 (1949).

(18) Kolle, W. and Gotschlich, E.: *Ztsch. f. Hyg. 44,* 1-28 (1903).

(19) Landois, L.: "Die Transfusions des Blutes" Leipzig, (1875).

(20) Landsteiner, K.: "The Specificity of Serologic Reactions" Harv. Press, (1945).

(21) Lovell, R.: *J. Comp. Path. 45,* 27 (1932).

(22) Lovell, R.: *J. Comp. Path. 47,* 107 (1934).

(23) Ludke, H.: *Centralblatte f. Bakt. 42,* 69 (1906).

(24) Mackie, T. J., and Finkelstein, M. H.: *J. Hyg. Camb. 28,* 172 (1928); *30,* 1 (1930).

(25) Mackie, T. J., and Finkelstein, M. H.: *J. Path. Bact. 37,* 359 (1933).

(26) Mamlock, L.: *Archiv. f. Hyg. 68,* 95 (1909).

(27) Powell, H. M.: *Amer. Jour. Hyg. 5,* 228 (1925).

(28) Preisz, H. R.: "Infectio es Immunitas" Budapest, (1936).

(29) Ramon, G., and Richou, R., *Compt. Rend. Soc. Biol. 121,* 621 (1936).

(30) Ramon, G., and Richou, R., Djourichitch, M.: *Rev. Imm. Paris 2,* 375 (1936).

(31) Reymann, G. C.: *J. Immunol. 5,* 227 (1920).

(32) Walsh, L. S. N.: *J. Immunol. 45,* 229 (1942).

(33) Wright, G. P., and Clark, W. M.: *Brit. Med. Jour. 2,* 146 (1944).

PREPARATION OF A RELATIVELY ANTIOXIDANT-FREE VEGETABLE OIL *

By Gordon S. Fisher ** and W. G. Bickford

IT is frequently desirable to have available fatty substrates for use in determining the antioxidant activity of various substances and for differentiating between antioxidant and synergistic activity. Such substrates are useful in testing stabilizers for fat-soluble vitamins and pharmaceuticals which are frequently prepared in vegetable oil carriers. They are also useful in preparing vitamin-free fat diets for use in nutritional investigations. The use of such substrates has been reported in previous publications (2, 3, 4) of this laboratory and has resulted in various requests for the details of the method of their preparation. These are set forth below.

Natural antioxidants and color bodies are removed by adsorption on activated alumina and carbon. Refined and bleached oils, particularly peanut oils, are the preferred starting materials because this oil is normally light colored thereby reducing the amounts of adsorbents required. It has been found most convenient to prepare about 10 pounds of oil at a time and store the finished product under an inert gas at refrigerator temperature until needed. The apparatus and procedure described below is intended to handle this amount of oil.

An adsorption column, 4 ft. high and 65 mm. inside diameter, is assembled as shown in Figure 1. The appropriate length of Pyrex tubing is inserted in a Buchner funnel of suitable size, and packed with 150 Gm. of a mixture of activated carbon and filter aid [1] (1:2) at the bottom, 2 Kg. of 80 mesh alumina (freshly reactivated by heating at 200° C. for several hours) in the middle, and a 2- to 3-inch layer of anhydrous sodium sulfate at the top. The various layers are separated by glass wool separators to facilitate recovery of the adsorbents. All materials are added in dry form.

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1. Hyflo Super Cel and GFO Carbon have been found satisfactory for this purpose. However, these products are not recommended over other materials having equal properties.

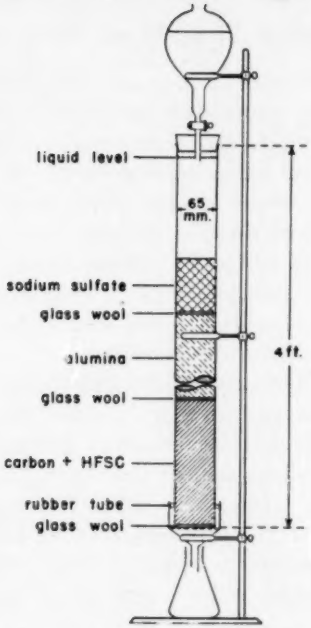

Fig. 1. Adsorption column for removal of antioxidants from vegetable oils.

About 10 lbs. of oil are dissolved in 10 liters of commercial pentane. The solution is poured into the top of the column and allowed to percolate through. A large separatory funnel may be used as a reservoir provided adequate precautions are taken to prevent leakage. The eluate is collected in about 1-liter portions and the spectral transmission of each fraction is measured by means of an Evelyn Colorimeter [2] using a No. 490 color filter. A mixture of pentane carbon tetrachloride (2:1) is used as a blank. Each portion is also tested for tocopherols by mixing 1 ml. of the eluate mixed with 1 ml. of a 0.1 percent solution of ferric chloride ($FeCl_3$) in ethanol, and 1 ml. of a 0.1 percent solution of α, α′ dipyridyl in ethanol and shaking for one minute. Development of a pink color indicates the presence of tocopherol.

2. This instrument is named in order to adequately describe the exact experimental procedure of the method. However. the Department of Agriculture does not recommend it over that of any other manufacturer of similar equipment.

All eluate portions having a negative tocopherol test and 98 percent or higher transmission are combined and used without further treatment with adsorbents. The other portions are numbered in consecutive order and passed through a second column in the same order. After all the oil solution has passed through the first column it is washed with about 20 liters of pentane until the transmission of the eluate is less than 75 percent or until the concentration of oil in the eluate is less than 5 percent. These washings are retained and used for washing a second column.

The second column is constructed in the same manner as the first but can be smaller, *e. g.* 50 mm. diameter, packed with 75 Gm. of the mixture of carbon and filter aid and 500 Gm. of alumina. Fractions are collected and tested as before and the satisfactory portions are combined with those from the first column. Any portions which are still colored or contain tocopherol may be passed through a third column in the same manner.

The oil is recovered from the eluate by removal of the pentane on a steam cone followed by stripping with an inert gas (preferably hydrogen) under a slight vacuum. For some uses it is desirable to deodorize the oil for about 2 hours at 200°C. in a glass deodorizer (1). The finished oil is flushed with the inert gas and stored in sealed brown bottles in the refrigerator until used.

From 10 pounds of oil, 6 to 8 pounds of colorless oil containing less than 0.001 percent of tocopherol is generally obtained. The finished oil usually contains about one-half of the unsaponifiable material present in the original oil. The fatty acid composition of the oil is practically unaltered by this treatment. For example, a peanut oil containing 31.0 percent linoleic acid as determined by the spectrophotometric method gave a colorless antioxidant-free substrate containing 30.3 percent linoleic acid. A peanut oil substrate prepared in this manner usually has a stability value of approximately two hours by the active oxygen method at 97.7°C.

Summary

A method for preparing essentially antioxidant-free and nearly colorless vegetable oils is described. The method comprises solution of an oil, preferably peanut oil, in pentane and passing it through a specially prepared adsorption column containing layers of activated carbon and filter aid, activated alumina, and anhydrous sodium sul-

fate. Peanut oil treated by this method yields a product having a keeping time of about 2 hours by the active oxygen method at 97.7°C. Such substrate oils are useful in testing and distinguishing between antioxidants and synergists, and in the preparation of vitamin-free fat diets.

LITERATURE CITED

1. Bailey, A. E. and Feuge, R. O., *Ind. Eng. Chem., Anal. Ed., 15,* 280-281 (1943).

2. Fisher, G. S., *Ind. Eng. Chem., Anal. Ed., 17,* 224-227 (1945).

3. Fisher, G. S., Kyame, Lillian, and Bickford, W. G., *J. Am. Oil Chemists' Soc., 24,* 340-343 (1947).

4. Swift, C. E., Mann, G. E., and Fisher, G. S., *Oil & Soap, 21,* 317-320 (1944).

DETECTION OF A FREE AMINO ACID IN FILTRATE OF E. COLI CULTURE MEDIUM BY PAPER CHROMATOGRAPHY

By Louis Gershenfeld and Alan Bernstein *

SOME investigators report that the intestinal organisms, especially *Escherichia coli,* contribute to the amino acid requirements of various animals (1, 2). However, it has not yet been clearly demonstrated whether the source of the amino acids is from the dead and disintegrated bacterial cells or from free amino acids produced by the living cells in excess of their own requirements.

Work done by other investigators, Camien, Salle, and Dunn (3), Stokes and Gunness (4), employed the microbiological method in determining the amino acid content of *E. coli* cells cultured on media containing complex peptones. Dunlop (5), cultured *E. coli* on two types of media. The first was a synthetic medium using diammonium hydrogen phosphate as the source of nitrogen and glucose as a source of carbon, while the second was a synthetic amino acid medium similar to the microbiological assay media. The amino acid content of the cells grown in these two media was determined microbiologically. Dunlop was unsuccessful in his attempt to demonstrate by microbiological methods the presence of amino acids in the concentrated filtrate of the phosphate-glucose medium. Polson (6) employed paper chromatography to demonstrate the amino acid content of *E. coli* cells grown on 0.5% casein digest agar. In this investigation, Polson did not describe any attempt to demonstrate the presence of amino acids, free or combined, in the filtrate of a liquid medium.

Purpose

The object of this investigation was to utilize paper chromatography as a means of determining whether or not free amino acids are present in the filtrate of an *E. coli* culture grown in a chemically defined medium.

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Experimental

A. *Preparation of Medium and Reagents*

1. *Medium*

The synthetic medium used was that formulated by Koser (7). The formula is as follows:

$NH_4H_2PO_4$	(Merck-Reagent)	1 gram
NaCl	(Merck-U. S. P.)	5 grams
$MgSO_4.7H_2O$	(Mallinckrodt-U. S. P.)	0.2 gram
K_2HPO_4	(Merck-Reagent)	1 gram
Glucose	(Merck-U. S. P.)	2 grams
Distilled Water		1,000 ml.

The reaction of the medium was adjusted before sterilization to pH 6.8 with N/1 NaOH. The reaction of the medium after sterilization was determined by use of a Beckman pH meter and found to be pH 6.68. Twenty milliliters of medium were dispensed in large round bottom centrifuge tubes and sterilized by autoclaving at 121° C. for 15 minutes.

2. *Sterile Acid Washed Sand*

Ordinary sand was allowed to remain in contact with potassium dichromate-sulfuric acid solution for 24 hours. The acid solution was removed by washing the sand several times with distilled water. Approximately one inch of sand was placed in a 250 ml. Pyrex beaker and sterilized by heating in an oven at 170° C. for 4 hours.

3. *Chromatographic Solvents*

The following solvents were prepared as described by Woiwod (8).

a. *n-Butanol-Acetic Acid Mixture*

The n-butanol-acetic acid mixture was prepared by adding 30 ml. of glacial acetic acid (Merck) to 250 ml. of 50/50 (V/V) mixture of n-butanol (Mathieson, Boiling Point range 116° to 118° C.) in a separatory funnel. After shaking, the mixture rapidly separated into two layers. The lower layer was removed and the volume was measured. If the volume was more or less than 90 ml., the solution was discarded.

The lower liquid layer was placed in a 250 ml. pyrex beaker at the bottom of the chromatographic chamber. The function of the aqueous layer (bottom layer) was to saturate the atmosphere of the chamber.

The "butanol-rich" layer (top layer) was placed in a large pyrex dish (14" x 8" x 1½") at the bottom of the chamber and used for running the chromatograms (see Experimental Method—D).

b. *Phenol-NH_3 Solvent*

The phenol-NH_3 solvent was prepared by adding 225 ml. of distilled water to 500 grams of phenol (Mathieson). The mixture was placed in a separatory funnel, shaken well, and then allowed to separate into two layers.

The "phenol-rich" layer (lower layer) was drawn off and the volume determined. One milliliter of 28% NH_4OH (Merck) was added to each 99 ml. of this lower layer to give a final concentration of approximately 0.3% (V/V) NH_3. This mixture was placed in a large Pyrex dish (14" x 8" x 1½") at the bottom of a second chromatographic chamber and used for running the chromatograms.

The aqueous layer (upper layer) was placed in a 250 ml. pyrex beaker at the bottom of the chamber. The function of the upper layer was to saturate the atmosphere of the chamber (see Experimental Method—D).

4. *Ninhydrin Reagent*

The 0.2% (W/V) ninhydrin reagent was prepared as follows:

Ninhydrin (triketohydrindine-Eastman Kodak)	1 gram
n-Butanol (Mathieson)	500 ml.

B. *Preparation of E. coli Cultures*

A strain of *E. coli* that was freshly isolated from human feces was used throughout this experiment. The organism was maintained on blood agar slants at 20° C. to 25° C., and transferred each week to another blood agar slant.

Prior to the seeding of the chemically defined medium, the organism was transplanted to 10 ml. of nutrient broth (Difco) and incubated for 18 hours at 37° C. The broth culture was centrifuged at 1500 rpm. for 15 minutes and the supernatant fluid was removed by aspiration. The bacterial cells were washed by resuspending the

sediment in sterile saline, again centrifuged, and the supernatant fluid removed. This process of washing was repeated five times. After the last washing, a sufficient amount of sterile saline was added to the sediment to obtain a turbidity of the same density as a #3 McFarland nephelometer tube (approximately 900,000,000 bacteria per ml.). One 4 mm. loopful of this suspension was seeded into each of six tubes containing 20 ml. of Koser's medium. The cultures were incubated for 18, 36 and 72 hours at 37° C. Duplicate cultures were made for each incubation period.

C. *Preparation of Cultures for Chromatographic Analysis*

Upon termination of the incubation period, the cultures were centrifuged at 200 rpm. for a half hour. The supernatant fluid of the cultures incubated for the same length of time was pooled aseptically after centrifuging and was then filtered bacteriologically using a Swinny filter with a Seitz pad to remove any remaining bacterial cells. The volume of the supernatant fluid was reduced to about 2 ml. by vacuum distillation at room temperature (20° C. to 25° C.) using aseptic technique. This concentrated filtrate was divided into two equal portions. One portion was chromatographed without further treatment. The other portion was hydrolyzed with 20 volumes of approximately 4% H_2SO_4 (Merck) for about 10 hours, neutralized with a saturated aqueous solution of $Ba(OH)_2$ (Baker-Adamson) and the volume was reduced to 2 ml. as described above.

The sedimented bacterial cells were washed with sterile saline five times to remove any adhering medium. The cells were fragmented by grinding them with sterile acid washed sand. A portion of the ground cells was chromatographed without further treatment. The remaining portion was hydrolyzed with approximately 4% H_2SO_4 and prepared for chromatography in the same manner as was the filtrate.

D. *Method of Chromatography*

Approximately 0.1 ml. of the filtrate and 0.1 ml. of the treated cells were chromatographed employing the two dimensional technique as described by Woiwod. Two glass panelled aquarium tanks (22" x 12" x 12") with petrolatum sealed glass plates were used as chromatographic chambers. The two solvents used were phenol-NH_3 and n-butanol-acetic acid mixture. The chromatograms were developed by spraying with a DeVilbiss #31 glass atomizer containing a 0.2% (W/V) solution of ninhydrin in n-butanol.

The relative Rf [1] values of nineteen amino acids (see table 1)

$$1.\ \text{Relative Rf value} = \frac{\text{absolute Rf value of substance}}{\text{absolute Rf value of proline}}$$

based on the absolute Rf [2] value for proline were determined for the

$$2.\ \text{Absolute Rf value} = \frac{\text{distance in cm. substance has traveled}}{\text{distance in cm. solvent has traveled}}$$

solvents and the experimental conditions used in this investigation. The determined absolute and relative Rf values of these amino acids are shown in table 1.

Duplicate specimens were chromatographed. One chromatogram had one loopful of proline added to the unknown material. Consequently, if proline was present in the unknown specimen, a characteristic yellow spot would appear on both chromatograms.

E. *Identification of Ninhydrin Reacting Materials on the Chromatograms*

The absolute and relative Rf values of the unknown spots were determined. By comparing these values to those of the known amino acids, the unknown spots were tentatively identified (see tables 1, 2, 3). Final identification was made by preparing two or more additional chromatograms of the same material (depending on the number of spots present) and to each, one loopful of one of the suspected amino acids was added. To serve as a control, one chromatogram had no known amino acid added. If, after the chromatogram was developed, it was found that the known amino acid spot was superimposed on the unknown spot, it was interpreted that the unknown spot was produced by the same known amino acid that was added. This procedure was performed for each unknown spot.

Findings

Only one spot was developed on the untreated and the hydrolyzed chromatograms of the filtrate of the 72 hour old culture of *E. coli*. Inasmuch as the same spot was present in both the untreated and the hydrolyzed chromatograms, the material producing the spot was undoubtedly an amino acid and not a peptide. This spot was identified (as described in Experimental Method—E) as leucine (see table 2). There were no apparent spots on the filtrates of the 18 and 36 hour old cultures.

TABLE I

ABSOLUTE AND RELATIVE RF VALUES OF KNOWN AMINO ACIDS

Amino Acid	Phenol NH_3 Solvent		n-Butanol-Acetic Acid Solvent	
	Range of Absolute Rf Values	Range of Relative Rf Values Based on Proline	Range of Absolute Rf Values	Range of Relative Rf Values Based on Proline
dl-Aspartic acid	0.16-0.17	0.186-0.197	0.19-0.25	0.543-0.714
dl-Glutamic acid	0.25-0.27	0.291-0.314	0.23-0.29	0.656-0.827
dl-Cystine	0.17-0.21	0.197-0.244	0.10-0.11	0.288-0.317
dl-Leucine	0.83-0.85	0.965-0.989	0.66	1.87
dl-Phenylalanine	0.82-0.85	0.955-0.989	0.64-0.65	1.83-1.86
dl-Norleucine	0.83-0.84	0.965-0.976	0.70	2.01
l-Histidine	0.56-0.60	0.650-0.697	0.14-0.15	0.398-0.428
dl-Isoleucine	0.80-0.85	0.930-0.989	0.62-0.66	1.77-1.87
dl-Threonine	0.49-0.51	0.569-0.594	0.25-0.27	0.714-0.771
dl-Serine	0.35-0.37	0.406-0.430	0.21-0.22	0.600-0.627
dl-Methionine	0.79-0.81	0.919-0.943	0.51-0.52	1.46-1.48
dl-Glycine	0.41-0.45	0.476-0.524	0.19-0.24	0.543-0.685
l-Hydroxyproline	0.67-0.68	0.779-0.790	0.23-0.26	0.656-0.744
l-Proline	0.86	1.00	0.35	1.00
l-Lysine	0.36-0.39	0.419-0.454	0.13	0.371
dl-Alanine	0.58-0.60	0.675-0.697	0.30-0.31	0.857-0.885
dl-Valine	0.76-0.78	0.884-0.906	0.51	1.46
l-Tryptophane	0.71-0.74	0.825-0.860	0.56-0.57	1.60-1.63
l-Arginine	0.39	0.454	0.16-0.19	0.456-0.543

TABLE 2

Rf Values of Unknown Spot on Chromatogram of Cell-Free Filtrate of a 72 Hour Old Culture of *E. coli* in a Glucose-Phosphate Medium as Compared to Rf Values of dl-Leucine

	Absolute Rf Values		Relative Rf Values Based on Proline	
	Phenol-NH_3 Solvent	n-Butanol-Acetic Acid Solvent	Phenol-NH_3 Solvent	n-Butanol-Acetic Acid Solvent
Unknown Spot	0.85	0.67	0.989	1.88
dl-Leucine	0.83-0.85	0.66	0.965-0.989	1.83-1.86

TABLE 3

Rf Values of Unknown Spots on Chromatogram of Acid Hydrolized Cell of *E. coli* Grown for 72 Hours in a Glucose-Phosphate Medium as Compared to the Rf Values of Various Known Amino Acids.

	Absolute Rf Values		Relevant Rf Values Based on Proline	
	Phenol-NH_3 Solvent	n-Butanol-Acetic Acid Solvent	Phenol-NH_3 Solvent	n-Butanol-Acetic Acid Solvent
Unkown Spot #1	0.84	0.66	0.980	1.84
dl-Leucine	0.83-0.85	0.66	0.965-0.989	1.83-1.86
Unknown Spot #2	0.37	0.12	0.444	0.369
l-Lysine	0.36-0.39	0.13	0.419-0.454	0.371
Unknown Spot #3	0.75	0.52	0.886	1.50
dl-Valine	0.76-0.78	0.51	0.884-0.906	1.46
Unknown Spot #4	0.40	0.20	0.456	0.545
l-Arginine	0.39	0.16-0.19	0.454	0.456-0.543

Chromatograms of the ground cells of *E. coli* of the 18, 36 and 72 hour old cultures did not show any distinct ninhydrin reacting spots, but rather a diffuse pigmentation along the edges of the solvent front. In all the chromatograms of the acid hydrolyzed cells there were four distinct colored spots. These spots were identified (as described in Experimental Method—E) as leucine, lysine, valine and arginine (see table 3).

The chromatograms of the concentrated uninoculated medium and the acid hydrolyzed uninoculated medium did not display any ninhydrin sensitive material.

Discussion

The presence of leucine in the cell-free filtrate of the 72 hour old culture of *E. coli* and its absence in the 18 and 36 hour old cultures suggests that the bacterial cells produce this free amino acid in excess of their requirements. Using microbiological methods, previous investigators of the amino acid content of *E. coli* cells have detected amino acids in the following decreasing order of abundance: leucine, lysine, valine, arginine, isoleucine, threonine, phenylalanine, methionine, histidine and tryptophane (3, 4, 5). However, it must be recognized that during prolonged incubation of cultures, autolysis of bacterial cells may occur and perhaps this free amino acid in the filtrate is a product of such action.

Block (9), and Gortner, Gortner, Jr., and Gortner (10), state that during the process of acid hydrolysis with a strong mineral acid, small amounts of amino acids are destroyed. This may account for the finding of only four amino acids (leucine, lysine, valine and arginine) in the bacterial cells of *E. coli* as found in this investigation as compared to the detection of eight to ten amino acids (leucine, lysine, valine, arginine, isoleucine, threonine, phenylalanine, methionine, histidine and tryptophane) in the cells of *E. coli* described by other investigators using both microbiological and chromatographic methods (3, 4, 5, 6).

Summary

1. A strain of *E. coli* freshly isolated from human feces was cultured in a liquid synthetic glucose-phosphate medium.

2. The cell-free filtrates of 18, 36 and 72 hour old cultures were concentrated and then chromatographed using phenol-NH_3 and n-butanol-acetic acid mixture as solvents.

3. The bacterial cells were ground with sterile acid-washed sand and then chromatographed.

4. Portions of the cell-free filtrate of the 72 hour old culture and the bacterial cells were hydrolyzed separately with acid and then chromatographed individually.

Conclusions

A free amino acid, namely leucine, was found in the cell-free filtrate of a 72 hour old culture (synthetic medium) of *E. coli*. It is probable that this amino acid is produced by the living cells (*E. coli*) in excess of their own needs or it may be the result of autolysis of the bacterial cells.

In addition to leucine, the acid hydrolyzed cells were found to contain lysine, valine and arginine.

BIBLIOGRAPHY

(1) Martin, G. J., Mixtures of Pure Amino Acids as Substitutes for Dietary Protein. *Proc. Soc. Expt'l. Biol. Med., 55,* 182-183 (1944).

(2) Hall, W. K. and Sydenstricker, V. P., The Production of Methionine Deficiency in the Rat with Low Casein Diets. *Arch. Biochem., 12,* 147-152 (1947).

(3) Camien, M. N., Salle, A. J., and Dunn, M. S., Investigations of Amino Acids, Peptides, and Proteins. XXII Percentages of Some Amino Acids in Lactobacilli. *Arch. Biochem., 8,* 67-78 (1945).

(4) Stokes, J. L. and Gunness, M., The Amino Acid Composition of Microorganisms. *J. Bact., 52,* 195-207 (1946).

(5) Dunlop, S. G., The Synthesis of Amino Acids by Escherichia coli in Pure Culture. *J. Bact., 58,* 457-462 (1949).

(6) Polson, A., Quantitative Partition Chromatography and the Composition of E. coli. *Biochemica et Biophysica Acta., 2,* 575-578 (1948).

(7) Koser, S. A., Utilization of the Salts of Organic Acids by the Colon-Aerogenes Group. *J. Bact., 8,* 493-520 (1923).

(8) Woiwod, A. J., A Technique for Examining Large Numbers of Bacterial Culture Filtrates by Partition Chromatography. *J. Gen. Microbiol., 3,* 312 (1949).

(9) Block, R. J. and Bolling, D., The Amino Acid Composition of Proteins and Foods, Springfield, Illinois, Charles C. Thomas, (1947).

(10) Gortner, R. A., Gortner Jr., R. A., and Gortner, W. A., Outlines of Biochemistry, New York, John Wiley and Sons, Inc., (1949).

SELECTED ABSTRACTS

Long Term Tetanus Immunization. Bigler, J. A. *Am. J. Dis. Child. 81*:226 (1951). A study over a period of ten years has revealed several interesting facts relative to the level of immunity produced by tetanus toxoid. The study was performed on 300 infants and children who were immunized between 1938 and 1940. During the 10-year period which followed, a total of 1,363 titrations of the blood serum for tetanus antitoxin were made on 262 of the children.

The study has revealed that a basic immunization can be obtained with either two or three initial injections of tetanus toxoid but, that protective levels are not always obtained from two injections. The more injections given the longer protective levels are maintained. All immunized children showed detectable levels of antitoxin in the blood serum up to 10 years following immunization. When booster injections were given there was a rapid response with the development of protective levels of antitoxin within seven days. The author also reported that protective levels developed quickly after each of as many as five separate booster injections.

As a result of this study the author concluded that although protective levels of antitoxin may be present in many cases it would seem advisable to give booster injections to immunized persons following any injury from which tetanus may be considered likely to develop.

Therapeutic Effect of Thiosemicarbazones in Experimental Corneal Tuberculosis. Rees, R. J. W., and Robson, J. M. *Brit. J. Pharmacol. 6*:83 (1951). The injection of about 1000 viable organisms of a bovine strain of *Mycobacterium tuberculosis* into the cornea of mice again proved to be a satisfactory method for the screening of antituberculous activity of chemotherapeutic com-

pounds as assessed by the effect on the incubation period and upon the type and development of the corneal lesions appearing after the incubation period, according to the authors. The normal incubation time for the appearance of tuberculous ulcers in the cornea of untreated animals under these experimental conditions is between 12 and 14 days.

The compound 4-ethylsulfonylbenzaldehyde thiosemicarbazone was given to 52 mice in a dose of 0.085 per cent thoroughly mixed with the powdered diet, beginning 24 hours prior to inoculation, and continued for a standard treatment period of 28 days. Forty-eight of these animals developed lesions with a mean incubation period of 43.8 days as compared with a mean incubation period for untreated controls of 13.3 days. Therefore, the treated mice developed lesions 15.8 days after discontinuation of treatment. From these results it would appear that the thiosemicarbazone has a bacteriostatic effect on these bacteria but that once the drug is eliminated from the animals the bacteria develop a lesion with an incubation period and a subsequent rate of progress identical with that seen in untreated mice.

A combination of 2 per cent *p*-aminosalicylic acid in the diet along with the thiosemicarbazone did not show any better results than that obtained with the thiosemicarbazone alone.

A combination with the thiosemicarbazone of 2 mg. of streptomycin injected subcutaneously night and morning (on Saturdays and Sundays only the morning dose was given) showed a definite additive effect. With streptomycin alone there were 11 mice, 4 of which developed corneal lesions between the 18th and 25th days, 6 between the 32nd and 46th days, and 1 was free of any ulcer when the experiment was terminated 168 days after inoculation. Some of the lesions, however, remained atypical. Ten mice were given the combined streptomycin and thiosemicarbazone treatment. Only 3 of the mice in this group developed lesions during the 168 days of the experimental period. Of these 3 one appeared on the 46th day and remained small and atypical and the other 2 appeared on the 49th day and developed into typical acute lesions. Thus the combination of streptomycin and 4-ethylsulfonylbenzaldehyde thiosemicarbazone produced an effect above that obtained with either of the compounds alone. According to the authors this additive effect was even greater than that previously reported using the same test method with a combination of streptomycin and *p*-aminosalicylic acid.

The Enhancement of the Effect of Oral Penicillin by the Use of Oil Containing Aluminum Resinate. Hsu, Y. C. and Buttle, G. A. H. *Brit. J. Pharmacol. 6*:89 (1951). Previous studies had shown that oils may be used as protective vehicles for the administration of oral penicillin. Since oils are not miscible with water and are absorbed in the intestine where penicillin also is absorbed to a maximum extent, it was felt that oils might carry the penicillin through the stomach into the intestine for absorption. The authors also felt that a water-repelling substance might enhance the effective absorption.

The authors verified the previous finding that if penicillin is given orally with an absorbable oil, such as cod liver oil, olive oil, or arachis oil, an increased blood level is obtained but if a non-absorbable oil such as liquid petrolatum is used the blood levels are no better than those obtained in the control animals.

The study, however, primarily deals with the effect of aluminum resinate on the blood levels of penicillin. This substance is a water-repelling substance that loses this property in an alkaline medium. It was definitely found that in mice, rats, guinea pigs, and rabbits, but not in cats, that aluminum resinate increased the blood level of penicillin when added to an oral preparation of penicillin in arachis oil. Mice infected with a strain of hemolytic streptococci were also protected to a greater degree when aluminum resinate was added to the penicillin preparation. In fact, the protection afforded was slightly greater than that obtained from an equivalent amount of procaine penicillin injected intramuscularly.

The mechanism of action of aluminum resinate is not fully known but three possibilities were suggested by the authors. The substance may increase the protective effect of oil on penicillin in the stomach by acting as a water-repelling substance, it may promote absorption of the penicillin or increase its stability in the intestine, or it may delay renal clearance in a manner similar to that of caronamide.

The study also included an investigation of aluminum abietate since abietic acid is the chief constituent of the resinate radical. The authors found that the enhancement of activity by aluminum abietate was of the same order as that of aluminum resinate, as was the toxicity. The toxicity found for aluminum resinate in mice was of the order of an LD50 of 8.75 Gm. per Kg. Thus it would seem that the abietic acid molecule is that which is essential to this action.

ACTH in the treatment of Erythroblastosis Fetalis. Simpson, J. W., Akeroyd, J. H., Swift, F. L., and Geppert, L. J. *U. S. A. F. Med. J. 2:207* (1951). Replacement transfusion is now recognized as the treatment of choice in erythroblastosis fetalis. Although the authors stated that they had had excellent results with this procedure they pointed out some of its limitations and dangers. Technically replacement therapy may not be available and even though available it may fail even in the most experienced hands. Even though replacement is 90 per cent complete the remaining antibody is not altered and the antigen-antibody reaction is only quantitatively reduced. In addition, the prolonged surgical procedure imposes a severe stress on the newborn infant.

The authors felt that ACTH might help the infant to better withstand the severe stress of replacement transfusion and they, therefore, treated two erythroblastotic infants with ACTH.

The first infant was given 12.5 mg. of ACTH one hour after delivery and 320 cc. of blood was given. The following morning 35 cc. of packed red blood cells was given. ACTH was given in doses of 12.5 mg. per day for 4 days and then 8.3 mg. per day for 3 days. The hemoglobin rose 6.5 to 16 Gm. per 100 cc., the nucleated cell count dropped from 100,000 to 15,600 by the 5th day, the percentage of normoblasts dropped from 50 per cent to zero by the 5th day and at 7 days the jaundice was markedly reduced. Clinically the infant did well.

The second infant received no blood but did received 12.5 mg. of ACTH at 12-hour intervals for 3 days, then 8.5 mg. at 8-hour intervals for 2 days, and finally 6.25 mg. every 8 hours for 40 hours. The initial dose of ACTH produced a drop in eosinophilis from 460 to 260 per cu. mm. in 4 hours, indicating good adrenal response. The erythrocyte count increased and stabilized at 3,500,000 and the hemoglobin at 12 Gm. per 100 cc. The Coombs developing test gradually decreased to a trace of adsorbed antibody by the 7th day and the nucleated cell count dropped to normal by the 7th day. Clinically the infant did extremely well.

Although conclusions cannot be drawn from two cases the results were sufficiently encouraging to warrant further trial.

BOOK REVIEW

Chymia. Volume 3. University of Pennsylvania Press, 3436 Walnut Street, Philadelphia. 1950. 251 pages. Illustrated. $4.50.

This is the third volume of annual studies in the history of chemistry printed under the auspices of the Edgar F. Smith Memorial Collection of the University of Pennsylvania. Henry M. Leicester, of San Francisco, is editor-in-chief. Each chapter has its own author or authors.

The subjects are diverse, and sometime rather obscure, but each topic is well handled by its author and usually contains data and compilation of facts not ordinarily found elsewhere. To indicate the wide variety of subjects, let us list the chapter titles: "Tenney Lombard Davis and the History of Chemistry", "The Beginning of Chemical Instruction in America", "Les Appareils D'Experimentation de Lavoisier" (this chapter in French), "Bio-Active Substances in the Nineteenth Century", "La Quimica en Los 'Elements de Chimie' de Orfila" (in Spanish), "Early American Chemical Societies" (by Wyndham Miles, a graduate of the Philadelphia College of Pharmacy and Science), "The Invention of Printing and the Diffusion of Alchemical and Chemical Knowledge", "Wandlungen in der Geschichtlichen Betrachtung der Alchemie" (in German), "Boyle and Bayle, the Sceptical Chemist and the Sceptical Historian", "Karl Friedrich Mohr, Father of Volumetric Analysis", "Henri Sainte-Claire Deville", "Bunsens Vorlesung Ueber Allgemeine Experimentalchemie" (in German), "The Owl of Heinrich Khunrath".

Unusual and diverting reading, and excellent reference material are provided by this book. Scholars are invited to submit original, unpublished contributions of this type for future volumes of "Chymia".

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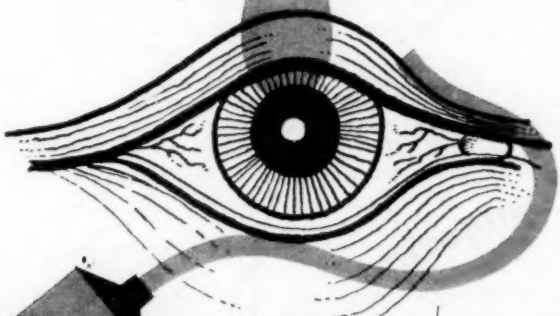

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